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Wheat Germ Oil Ameliorates Sucrose Induced Hepatosteatosis and Dyslipidemia through Controlling Lipogenic and Cholestrogenic Genes Expression in Rats

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***Abstract* – Sucrose high diets induce hepatosteatosis and dyslipidemia while wheat germ oil has been shown to relive them. Thus this work was carried to evaluate the possible mechanisms through which wheat germ oil attenuate sucrose induced hepatostiatosis, and dyslipidemia in rats. Twenty eight male Wister albino rats were divided into 4 groups. Control group was received tap water, 2nd group was received 60% sucrose in drinking water, 3rd group was supplemented with wheat germ oil (1.5ml/kg.BW/day orally) and 4thgroup was given 60% sucrose in drinking water and supplemented with wheat germ oil (1.5ml/kg.BW/day orally) simultaneously for 30 days. Sucrose administration in rats induced hepatosteatosis, dyslipidemia and hyperglycemias. Sucrose increased blood glucose level, serum levels of triacylglycerol, total cholesterol, VLDL-c and LDL-c and mRNA expression of pyruvate kinase, hydroxymethyl glutrayl CoA reductase, and fatty acid synthase as well as it induce fatty change in hepatocytes while it decreased serum level of HDL-c. In contrast, supplementation of sucrose administrated rats with wheat germ oil enhanced hemoglobin concentration and hematocrit value and normalized the altered lipid profiles through normalizing mRNA expression of hydroxymethyl glutrayl CoA reductase, and fatty acid synthase. In addition it induced hypoglycemia through increasing mRNA expression of pyruvate kinase and reducing mRNA expression of pyruvate carboxylase as well as it restored the histological structure of hepatic tissue. This study indicated that wheat germ oil protected rats against sucrose induced hepatosteatosis and dyslipidemia through controlling lipogenic and cholestrogenic gene expressions suggesting that wheat germ oil is a promising agent against sucrose induced hepatostiatosis and dyslipidemia.**

***Keywords* – Hepatosteatosis, Dyslipidemia, Wheat Germ Oil, Sucrose.**

I. INTRODUCTION

Nonalcoholic steatohepatitis (NASH) is a health-threatening disease [1] ranging from simple hepatic steatosis (accumulation of triglyceride in hepatocytes) to non alcoholic steatohepatitis (hepatic inflammation and necrosis) [2]-[3]. Cholesterol plays a major role in the development of NASH [4] which, associated with dyslipidemia [5].

Dyslipidemia is an abnormal elevation of plasma lipids including, total cholesterol, low density lipoprotein cholesterol (LDL-c) and triacylglycerol (TG) or low levels of high density lipoprotein cholesterol (HDL-c) [6].

Sucrose is a caloric sweetener and represents about 10-25% of total energy intake in most parts of the world [7]. Consumption of a high sucrose diet results in obesity, insulin resistance, diabetes, dyslipidemia, fatty liver, atherosclerosis and hypertension [8]-[9] because sucrose enhances hepatic lipogenesis [10]. Sucrose molecule is composed of one molecule of glucose and one molecule of fructose. Fructose derived from sucrose is converted into trioses in the liver that used for the de novo synthesis of TG and cholesterol inducing hyperlipidemia, decreased insulin sensitivity, and increased visceral adiposity in overweight/obese adult [11]-[12]. Moreover, feeding rats on sucrose rich diets results in fatty liver [13], hepatic oxidative stress [14] and consequently, hepatic injuries and elevates serum levels of TG, total cholesterol, LDL-c, glucose, liver function biomarkers enzymes and insulin while it decreases serum HDL-c level [15]. Thus, natural antioxidant and lipotropic agents became of great importance to alleviate sugar induced hepatic steatosis.

Wheat germ oil is one of natural products extracted from the germ of the wheat kernel. It is a good source of antioxidants including carotenoids, tocopherols, flavonoids, phenolic acids and α-tocopherol (vitamin E) which represents around 60% of the total content [16]-[17]. These ingredients enable wheat germ oil to have free radicals scavenging activity and protect rats against clozapine-induced hepatic oxidative stress [18]-[19]. In addition, Wheat germ is a good source of phytosterol mainly β sistosterol and octacosanol which regulate blood sugar and insulin levels in Type II diabetics by stimulating the release of insulin, inhibiting glucose -6- phosphatase and lowering plasma cholesterol level respectively [20]-[21]. Further, Wheat germ oil increases serum levels of HDL-c while reduces serum level VLDL-c and the harmful effects of LDL-c [22]-[23]. Thus, wheat germ oil has regulatory effects on hepatic lipid metabolism through stimulating β oxidation of fat and suppressing fatty acid synthesis which consequently reduce hepatic fat accumulation [24]. Subsequently wheat germ oil plays important roles in regulation of fatty liver and dyslipidemia. Therefore, this study was carried out to assess the possible mechanisms through which wheat germ

oil relives hepatic steatosis and dyslipidemia in rats fed on high sucrose diet.

II. MATERIALS AND METHODS

A. Chemicals and Diagnostic kits:

Sucrose and wheat germ oil (WGO) were purchased from Mid Egypt Chemical Company, and El-Hawag factory, Badr City, Egypt respectively. TRIzol (easy RED^{TM} total RNA Extraction Kit, iNtRON Biotechnology, Inc.), Ethanol 97%, chloroform, Isopropanol, Maxime RT PreMix Kit, (iNtRON Biotechnology, Inc.), primers and Real MOD^{TM} Green Real-time PCR Series (iNtRON Biotechnology, inc.) were obtained from Biovision chemical company, Egypt. Kits for hemoglobin estimation obtained from Diamond Diagnostics, Elgomhoria Company, Egypt. Kits for assaying blood glucose level and serum levels of TG, total cholesterol, HDL-c, alanine amino transferase (ALT) and aspartate amino transferase (AST), were purchased from Spinreact (Girona, Spain).

B. Animals

A total of twenty eight male Wister albino rats (weighting 100-120 gm) were used in this experiment. Rats were purchased from Al-Zyade experimental animal production center, Giza, Egypt. The animals were housed in polyethylene cages, with stainless steel wire lids (bedded with wood shavings), and kept under a standard laboratory conditions of temperature (20-25 °C) and 12 h light/dark cycle. The animals were allowed a balanced ration. Water and feed were supplied ad libitum. All experimental design and conditions were approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt. The animals were quarantined for 10 days before the beginning of the experiment for acclimatization.

C. Experimental design

Rats were randomly divided into four experimental groups seven animals each (n=7). Rats of the 1stgroup were given tap water and feed ad libitum throughout the experiment and kept as a control. Rats of the 2nd group were received 60% sucrose [15] in drinking water. Rats of the 3rdGroup were supplemented with wheat germ oil (1.5ml/kg.BW/day orally) [25]. While animals of the 4th group were given 60% sucrose in drinking water and supplemented with wheat germ oil (1.5ml/kg.BW/day orally) simultaneously.

D. Blood sampling:

At the end of the experimental period, 30 days, blood samples were collected from retro-orbital puncture after diethyl ether anesthesia. Blood samples were divided into two parts. One part was drawn into dry sodium fluoride containing tubes for measuring of blood glcucose level. While the other part was taken in dry tubes, kept at room temperature for 30 minutes. Sera samples were separated and kept at -20°C for subsequent analysis.

E. Tissue sampling:

Liver specimens from rats were taken and kept at −80 °C until used for analysis of gene expression of some lipogenic, glycolytic and gluconeogenic enzymes, hydroxyl methyl glutrayl COA (HMG CoA), fatty acid synthase (FAS), pyruvate kinase (PK), pyruvate carboxylase (PC) and β actin. Other specimens from the liver were fixed in 10% neutral buffered formalin for histopathological examination.

F. RNA extraction and cDNA synthesis:

Total RNAs was isolated from liver tissue by using TRIzol reagent (easy REDTM Total RNA Extraction Kit, iNtRON Biotechnology, Inc.). cDNA was synthesized by using Maxime RT PreMix Kit. One micro gram of RNA was used in a reaction volume 20 µl in the Maxime RT PreMix tubes. cDNA synthesis reaction was performed at 45^{o}C for 60 minutes and RTase inactivation was done at 95^{o}C for 5 minutes in thermocycler.

G. Hematological examination:

Hemoglobin (Hb) concentration was determined by the Cyanomethemoglobin Method using Drabkin's solution [26]. Ferricyanide oxidizes the hemoglobin to methemoglobin. The methemoglobin reacts with cyanide ions to form cyanomethemoglobin which can be measured spectrophotometrically. Packed cell volume (PCV) was determined by micro hematocrit method as described by Feldman et al. [27] using microhematocrit centrifuge.

H. Chemical assay:

Serum glucose level was measured according to Young [28] as the oxidation of glucose into gluconic acid and H_2O_2 was catalyzed by glucose oxidase and evolved H_2O_2 reacts with the chromogenic oxygen receptor, phenol, and 4-aminophenazone in the presence of peroxidase to form chromogenic quinoneimine. Serum TG level was measured by using the enzymatic colorimetric method of Fassati and Principe [29]. Lipase enzyme hydrolyze TG producing glycerol and fatty acids. Glycerokinase catalyzes of the reaction between glycerol and ATP to produce glycerol-3-phosphate and ADP. Glycerol-3-phosphate oxidase converts glycerol-3-phosphate into dihydroxyacetone phosphate and H_2O_2. H_2O_2 reacts with 4-chlorophenol and 4- aminoantipyrin to produce quinoneimine by an action of peroxidase that can be measured calorimetrically. Serum total cholesterol level was assessed using the enzymatic colorimetric method of Allain et al. [30]. Serum cholesterol ester was enzymatically hydrolyzed and oxidized. The quinoneimine formed from H_2O_2 and 4-aminoantipyrine in the presence of phenol and peroxidase can be measured spectrophotometrically. Determination of HDL-c level was carried out according to Grove [31]. LDL-c and Very low-density lipoproteins cholesterol (VLDL-c) were calculated according to Friedewald [32]. The activities of serum AST and ALT were estimated according to Young [28]. ALT and AST catalyze the transfers of the amino group of glutamic acid to pyruvic acid and oxaloacetic, respectively, the enzyme activity is proportional to the amount of pyruvate and oxalate formed over a definite period of time and is measured by a reaction with 2, 4-dinitrophenylehydrazine in alkaline solution yielding the corresponding colored compound, which can be measured spectrophotometrically.

J. Analysis of HMG COA, FAS, PK and PC gene expression in rats' hepatic tissues:

Table 1: Primer list and quantitative real time PCR conditions

Gene (Accession No)	Forward primer sequence Reverse primer sequence (PCR product size) [Annealing conditions]
Rat PK (BC161827)	5`-ATGATGTGGATCGAAGGGTC -3` 5`- TGGGTTGAAAGAAATAGGGT -3` (+930 to + 1129, 200 bp) [56 °C, 30s, 40 cycles]
Rat PC (BC085680)	5`- CCCTGTGGACCCCATTGTT -3` 5`- TCGCAGAAGGATGTCTCTGAAA -3` (+1612 to +1684, 73 bp)[60 °C, 30s, 40 cycles]
Rat HMG-CoA (X55286.1)	5`-GATTTCCAAGGGTACGGAGA-3` 5`-TTATGGCAGCAGGTTTCTTG-3` (+2004 to +2116), 114bp)[60 °C,30s, 40 cycles]
Rat FAS (NM_017332)	5`- AGGGGTCGACCTGGTCCTCA -3` 5`- GCCATGCCCAGAGGGTGGTT -3` (+5285 to +5416, 132 bp) [60 °C, 30s, 40 cycles]
Rat β-actin (XM_006248886)	5`- GATTACTGCCCTGGCTCCTA -3` 5`- TCATCGTACTCCTGCTTGCT -3` (+387 to +530, 150 bp) [56 °C, 30s, 40 cycles]

To estimate mRNA expression of genes in different groups, quantitative real time PCR was performed. cDNA, which was reverse transcribed from total RNA, was used as a template for quantitative real time PCR. The oligonucleotide primers of HMG CoA, FAS, PK and PC for quantitative real time PCR have been described in Table (1). The Ct value was determined, and the abundance of gene transcripts was calculated from the Ct value by normalizing against β actin.

K. Histopathological examination of hepatic tissue:

Liver specimens were taken, washed in saline and preserved in 10% neutral buffered formalin for histopathological studies. After 72 h of fixation, samples were dehydrated, embedded in paraffin wax and sectioned (3µm) for haematoxylin and eosin (H&E) staining according to Bancroft and Stevens [33].

M. Statistical analysis:

Data were expressed as mean ± SE and analyzed by using a one-way analysis of variance (ANOVA) with Duncan's post hoc test to determine the significant differences among data in this study. The differences between means were analyzed at the 5% probability level ($P \leq 0.05$) was considered statistically significant.

III. RESULT

A. Supplementation of rats with wheat germ oil enhances hemoglobin concentration and hematocrit value in rats:

Effects of sucrose administration and/or WGO supplementation in rats are presented in Table 2. Supplementation of rats with WGO (3rd group) for 30 days significantly increased Hb concentration (p<0.05) compared to control (1st group). However, administration of rats with sucrose has no significant effect on Hb content either alone or together with WGO (2nd and 4th groups), respectively. In addition WGO significantly enhanced PCV in the 3rd group (p<0.05) compared to control and sucrose administrated group (1st and 2nd groups), respectively.

B. Supplementation of rats with wheat germ oil normalized the serum metabolites altered by sucrose administration

The effects of sucrose administration and protective effects of WGO on blood glucose level, serum lipid profile and serum enzymatic activities of liver function biomarkers are shown in Table3. Administration of rats with sucrose (2nd group) in drinking water for 30 days significantly elevate (p< 0.05) blood glucose level and serum levels of total cholesterol and LDL-c compared to those of the 1st, 3rd and 4th groups and serum levels of TG and VLDL-C in comparison to those of the 1st and 3rd group. In addition, it significantly elevates the activity of serum AST compared to 3rdgroup. However, it numerically reduces serum level of HDL-C compared to control and WGO supplemented groups. In contrast, supplement of rats with WGO alone or in concernment with sucrose (3rd and 4th group), respectively significantly decrease (p< 0.05) blood glucose level compared to 1st and 2nd group. In addition, it significantly (p< 0.05) reduce the activity of serum AST and the serum levels of TG, total cholesterol, LDL-c and VLDL-C of the rats of the 3rd

Table 2: Effect of wheat germ oil supplementation on hemoglobin concentration and hematocrit value of control and sucrose treated rats

Parameters	Control	Sucrose	WGO	Sucrose &WGO
Hb (g/dl)	12.28 ±0.54 ^{b}	12.76±0.21 ^{ab}	13.37±0.24 ^{a}	12.37±0.28 ^{ab}
PCV (%)	33.3±0.4 ^{c}	34.8±0.5 ^{bc}	37.2±0.9 ^{a}	36.5±0.3 ^{ab}

Data are expressed as means ± SE

Values carrying different letters in the same row are significantly different at P< 0.05

Table 3: Effect of wheat germ oil supplementation on serum biochemical parameters of control and sucrose treated rats

Parameters	Control	Sucrose	WGO	Sucrose & WGO
AST (IU/L)	46.1±0.7 [ab]	49.3±0.9 [a]	44.7±0.9 [b]	48.7±1.1 [a]
ALT (IU/L)	69.6±0.8	69±1.1	71.9±2.4	72.9±1.0
Glucose (mg/dl)	95.7±3.7 [b]	105.6±3.2 [a]	78.4±2.2 [c]	81.8±2.3 [c]
TG (mg/dl)	157.6 ±12.9 [b c]	188.9±8.5 [a]	160.4±4.7 [c]	181.1±1.5 [ab]
CHO (mg/dl)	177.4±5.8 [b]	227.3±8.4 [a]	181.4±4.8 [b]	187.5±3.1 [b]
HDL-c (mg/dl)	43.9±5.0	41.6±2.4	47.2±0.6	46.0±0.9
LDL-c (mg/dl)	101.98±2.6 [b]	147.92±5.3 [a]	102.12±4.2 [b]	105.28±3.1 [b]
VLDL-c (mg/dl)	31.52±1.2 [bc]	37.78±1.7 [a]	32.08±1.0 [c]	36.22±0.6 [ab]

Data are expressed as means± SE
Values carrying different letters in the same row are significantly different at $P< 0.05$

group compared to sucrose administrated group (2nd group). Moreover, WGO kept the activity of serum AST and serum levels of total cholesterol, LDL-c and VLDL-c within normal control range. While it slightly increase serum level of HDL-c in the 3rd and 4th groups compared to those of the 1st and 2nd groups. Administration of rats with sucrose and WGO separately or together had no significant effect on the activity of serum ALT.

C. Wheat germ oil protected rats' livers from sucrose induced fatty changes:

To confirm the protective effects of WGO against the adverse effects of sucrose on hepatocytes, we examined hepatic tissues grossly and microscopically for detection of any changes and abnormalities.

D. Gross appearance:

Liver of sucrose administrated group (2nd group) showed mild yellow-pale coloration but with normal shape features (Fig. 1B). No abnormal features could be observed in liver of other groups (Fig.1A, C, & D).

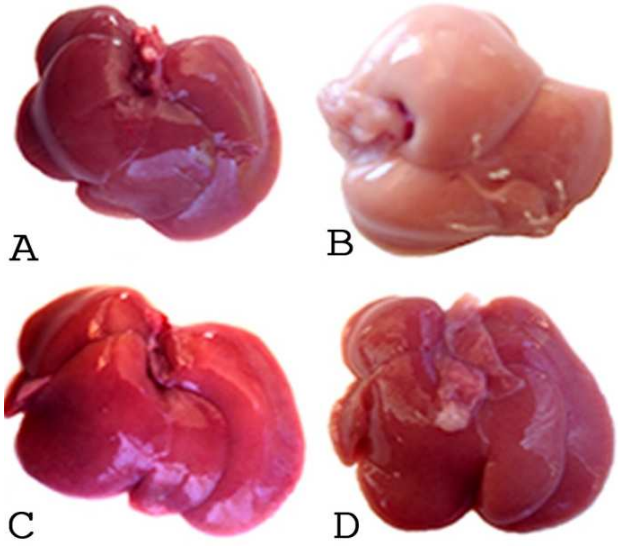

Fig.1. A, B, C, and D are gross liver pictures of control, sucrose supplemented, wheat germ supplemented, and sucrose plus wheat germ supplemented groups respectively: Liver from sucrose supplemented group showed mild yellow-pale coloration (Fig. 1: B). No abnormal features could be observed in liver of other groups (Fig. 1: A, C, & D).

E. Histopathological changes:

Hepatocytes of sucrose administrated group showed macrovesicular fatty changes in the cytoplasm and the nucleus become eccentric (arrows) (Fig. 2B). Hepatocytes of control, WGO supplemented groups and sucrose administrated rats supplemented with WGO (1st, 2nd and 4th group) respectively, appear with the normal histological architecture (Fig. 2 A, C & D).

F. Sucrose altered glycolytic, cholestrogenic and lipogenic enzymes gene expression while wheat germ oil reduced gluconeogenic, lipogenic and cholestrogenic enzyme gene expression:

To reveal the mechanisms through which WGO protect rats from the adverse effect of sucrose and kept the altered metabolites within normal range we evaluated gene expression of glycolytic, gluconeogenic, cholestrogenic and lipogenic enzymes in the control and different treatment groups. The effects of sucrose and WGO administration on the gene expression of glycolytic, gluconeogenic, cholestrogenic and lipogenic enzymes of rats' livers are illustrated in Fig.3. Administration of rats with sucrose and/or WGO (2nd, 3rd and 4th groups) significantly enhanced ($p< 0.05$) mRNA relative expression of glycolytic enzyme PK, one of the rate limiting enzymes of glycolysis, compared to that of the control group Fig. 3A. However, they significantly diminished ($p < 0.05$) the mRNA expression of gluconeogenic enzyme PC, one of the rate limiting enzymes of gluconeogenesis, in comparison to the control group Fig. 3B. Administration of rats of the 2nd group with sucrose of successive 30 days significantly increased relative mRNA expression of cholestrogenic gene HMG CoA reductase Fig. 3C and lipogenic gene FAS Fig. 3D compared to the control and other treated groups. However, supplementation of rats with WGO alone (3rd group) or in concernment with sucrose (4th group) diminished the mRNA expression of HMG CoA reductase in comparison to 1st and 2nd groups Fig. 3C and kept the expression of FAS of the 3rd and 4th groups within normal control range Fig. 3D.

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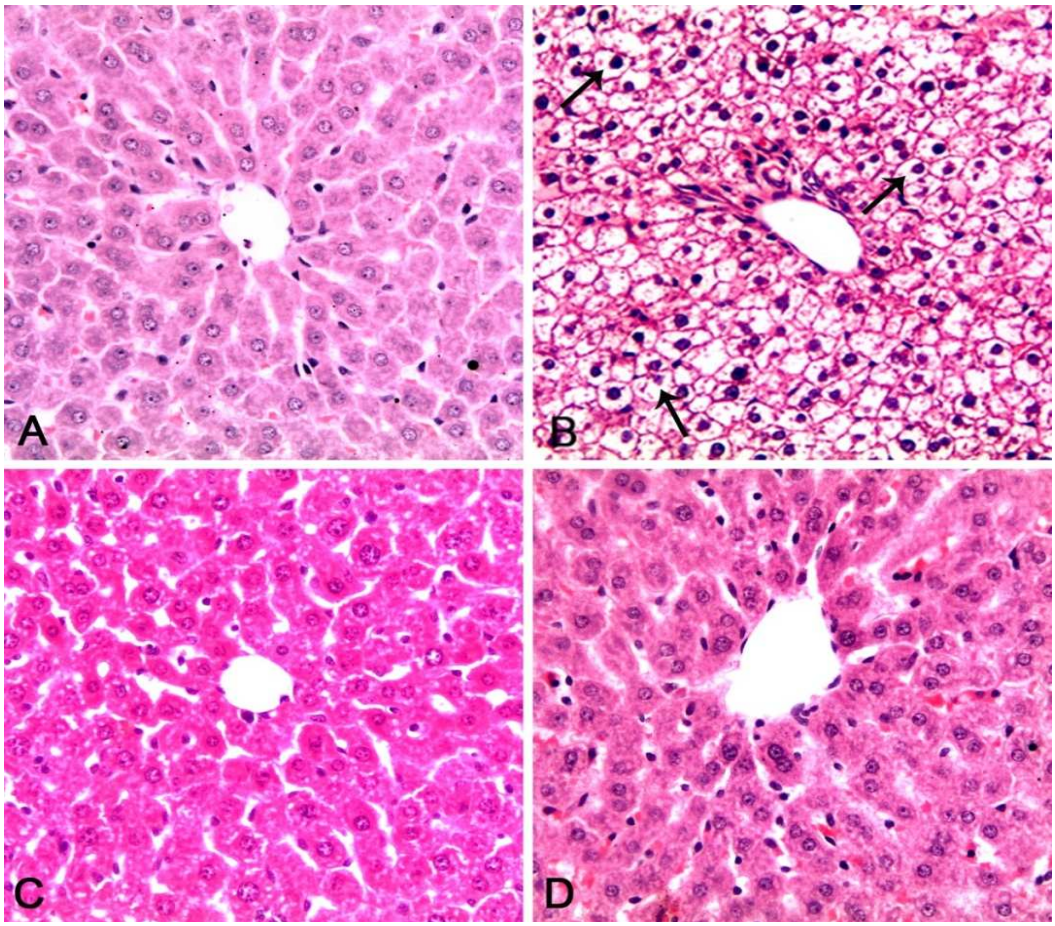

Fig.2. A, B, C, and D are histopathological pictures of control, sucrose supplemented, wheat germ supplemented, and sucrose plus wheat germ supplemented groups respectively: Comparative liver morphology revealed that some hepatocytes showing macrovesicular fatty changes and eccentric nucleus (arrows) (Fig. 2: B). No histological changes could be detected in other groups (Fig. 2: A, C, & D). Hematoxylin & eosin. X40.

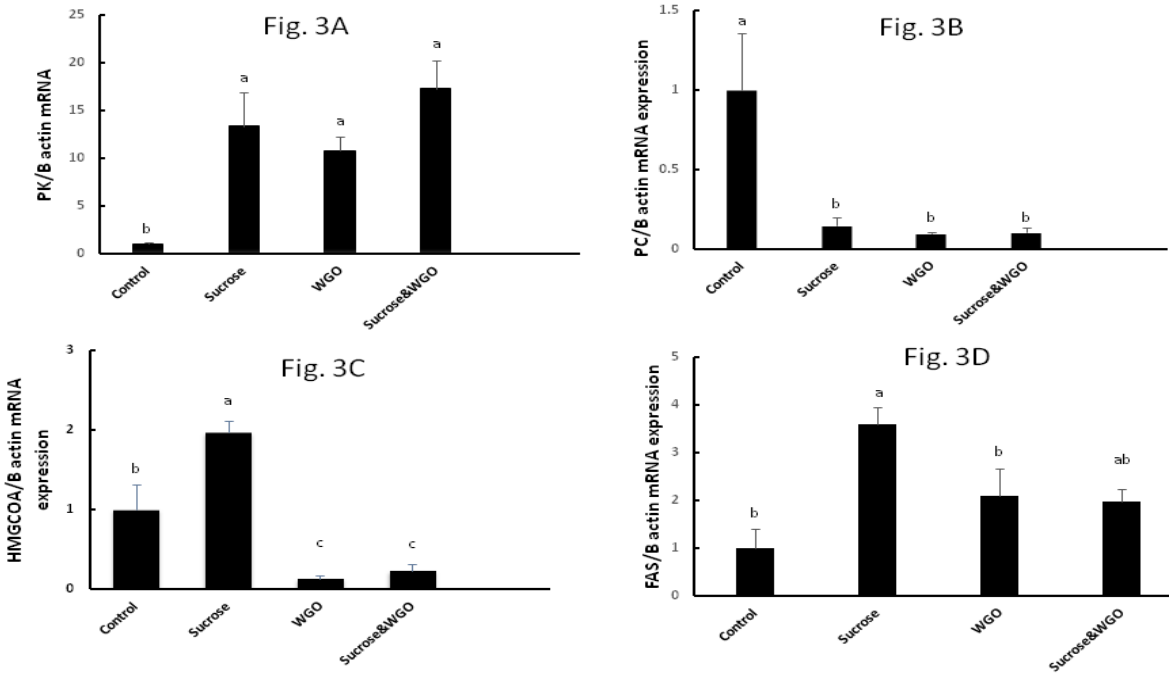

Fig.3. Expression of pyruvate kinase (PK), pyruvate carboxylase (PC), hydroxyl methyl glutrayl CoA (HMG-CoA) reductase and fatty acid synthase (FAS) in different group of rats. Columns having different letters are significantly different ($P< 0.05$).

IV. DISCUSSION

Total caloric intake may have a role in nonalcoholic fatty liver [34]. Ingestion of fructose especially from sweetened beverages is strongly associated with nonalcoholic fatty liver [35]- [36]. The major sources of fructose are sucrose and high fructose corn syrup [37] and administration of experimental animals with fructose, sucrose or high fructose corn syrup induces hepatic steatosis [38]- [39]- [40]. Hepatic steatosis is also induced in calorically restricted rats supplemented with high sucrose diet [39]. Moreover, administration of humans with fructose increased denovo lipogenesis and hepatic triglyceride storage [41]. NASH induces insulin resistance, oxidative stress, lipid peroxidation, cytokine production and innate immunity [42] -[43].

The present study indicated that supplementation of rats with WGO caused significant increase in Hb concentration and hematocrit value. These observations were in line with those of Barakat et al. [44] who reported that treatment of rats with wheat germ improved Hb concentration and PCV value that altered by chlorpyrifos administration. This improvement effect of WGO on hematological parameter can be explained by its antioxidant potential as it contains high amount of α-tocopherol (vitamin E). Vitamin E might be the most effective natural lipid soluble, chain breaking antioxidant, which scavenges free radicals, thus it prevents free radical- induced lipid peroxidation in the RBC membrane thereby decreases hemolysis and increases RBC survival and subsequently increased Hb concentration and PCV [45].

In addition, these results showed that administration of rats with sucrose altered the histological feature of the hepatic tissues because one of the histopathological features of nonalcoholic fatty liver disease is fatty change or steatosis of hepatocytes. Fatty change of hepatocytes either to be macrovesicular or microvascular. Macrovesicular steatosis is represented by large vacuoles occupying almost the entire cytoplasm and pushing the nucleus to the periphery of the cell. In microvesicular steatosis, multiple small lipid vacuoles are detected in the cytoplasm and the nucleus is usually located at the cell center [46]. In this study, WGO supplementation prevents the formation of macrovesicular fatty changes which appeared in hepatocyets of sucrose supplemented rats. Murase et al. [24] reported that WGO reduces hepatic lipid accumulation and suppresses fatty acid synthesis. Moreover, Morsy et al. [15] demonstrated that high sucrose diets increases serum levels of gamma glutamyl transferase, AST, ALT and alkaline phosphatase and alters the histological structure of liver tissue. These improvements in the liver function and structure may attribute to omega-3 fatty acids and linolenic acid present in WGO [47]. Therefore, WGO may play a role in reduction of fat accumulation inside hepatocyets.

Furthermore, Administration of rats with sucrose altered glucose, lipid and cholesterol metabolism inducing hyperglycemia and dyslipidemia. The hyperglycemia induced by sucrose in this study was in line with the finding of Kendig et al. [48] who reported that administration of female rats with sucrose 10% for four weeks produces hyperglycemia and glucose intolerance. Shambaugh et al. [49] added sucrose administration to experimental animals induces hyperglycemia and glucose intolerance because it reduces mRNA and protein expression and the activity of glucose transporter 4 in Sprague-Dawley rats' adipocytes, the main transporter of glucose in adipose tissue and skeletal muscles which consume the greater amount of glucose in the body [50]. However, supplementation of the rats with WGO either alone or in concernment with sucrose reduced blood glucose levels. The hypoglycemia induced by WGO supplementation in this study can be explained by increasing glucose oxidation and decreasing glucose production in the liver by regulating gene expression of the key enzymes that control glucose oxidation, glycolysis, and glucose production, gluconeogenesis. The current study indicated that WGO increased mRNA expression of hepatic PK (one of the rate limiting enzymes of glycolysis) and reduced expression of hepatic PC (one of the rate limiting enzymes of gluconeogenesis) to our best of knowledge it was interesting and novel finding. In addition, Megahad and El Kinawy [21] found that wheat germ regulates blood sugar and insulin levels in type II diabetics by stimulating the release of insulin and inhibiting glucose -6- phosphatase.

Dyslipidemia induced by sucrose administration in this study was in line with Yang et al. [51] who showed that hepatic content of triglyceride and cardiac content of rats fed sucrose-rich diet were elevated. In addition, feeding rats high sucrose diets elevated serum levels of glucose, insulin, TG, total cholesterol, LDL while HDL level was reduced as well as alters the histological structure of liver tissue [15]. Such finding may be explained by elevated gene expression of PK, FAS and HMG-CoA reductase that induced by sucrose administration in the current study. Elevated PK expression can increase the vicinity of pyruvate, the precursor of acetyl CoA which is the precursor for cholesterol synthesis. FAS, a multiunit enzyme complex that catalyzes the synthesis of long-chain fatty acids from acetyl CoA and malonyl CoA [52], controls the lipogenic capacity of the liver. HMG-CoA reductase is an endoplasmic reticulum–bound and peroxisomal enzyme, the rate-limiting enzyme in cholesterol biosynthesis, is abundantly expressed in the liver and plays a central role in regulation of cholesterol metabolism and plasma cholesterol concentration [53]. Carbohydrate feeding was found to increase the activity of these enzymes in the liver [54]. Thus, the elevated expression of these enzymes may contribute in the induction of dyslipidemia and hepatosteatosis in sucrose administrated rats as it was reported that ingestion of a diet high in sucrose induces rapid onset of hepatosteatosis possibly due to the rapid response of lipogenic, insulin signaling and inflammatory genes [55]. Dyslipidemia induced by administration of sucrose was inhibited by supplementation of sucrose administrated rats with WGO possibly due to either it kept the expression of FAS and HMG-CoA reductase within normal control ranges or it increases the membrane permeability and fluidity, which

decrease triglycerides, phospholipids and cholesterol levels [56]. In, addition, phytosterol content of WGO may also play a role as it decreases the absorption of cholesterol and reduces the expression of HMG-CoA reductase expression which consequently decreased serum levels of TG, phospholipids and cholesterol [57]-[58]-[59]-[60]. Moreover, WGO reduces hepatic lipid accumulation by stimulating its oxidation and suppressing fatty acid synthesis [24]. A causal relationship seemed to exist between hepatic FAS activity and serum TG concentrations. Both were increased by sucrose feeding and lowered by WGO supplementation. These results are consistent with those of Goodridge [61] who demonstrated that feeding rats a high carbohydrate diet increases FAS activity and lipogenesis in the liver while WGO supplementation lowered serum TG and normalized FAS activity in the liver. Finally, the possible cause of the ameliorative effect of WGO on sucrose induced dyslipidemia and hyperglycemia is the protective effects of WGO on the liver tissues as it inhibited the fatty changes in hepatocyets, the main sites for glucose metabolism, lipogenesis and cholesterol synthesis. Therefore, WGO has ameliorative effect on sucrose induced hepatostiatosis, dyslipidemia and hyperglycemia.

V. Conclusion

Sucrose administration induced hyperglycemia, hepatostiatosis and dyslipidemia in rats through alteration of glycolytic, lipogenic and cholestrogenic enzymes gene expression and histological structures of hepatic tissue. WGO ameliorates sucrose induced hyperglycemia, hepatostiatosis and dyslipidemia through normalizing gluconeogenic, lipogenic and cholestrogenic enzymes gene as well as restoration of the normal structure of hepatic tissue. This study suggested that WGO may be a promising protective agent against sucrose induced hepatostiatosis and dyslipidemia.

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